microsomal Ca-ATPase inhibitor 2,5-di-(*tert*-butyl)-1,4-hydroquinone in cystic fibrosis pancreatic epithelial cells, J. Clin. Invest. 96:1794-1801 (1995)).

Q. Pharmaceutical Preparations

General. The therapeutics compositions of this invention can be used in the form of a medicinal preparation, for example, in solid, semi-solid or liquid form which contains the composition of the present invention, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for external, enteral or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic pharmaceutically acceptable carriers for tablets, pellets, capsules, inhalants, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. Formulations of the present invention encompass those which include carriers such as water, talc, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, corn starch, keratin, colloidal silica, potato starch, urea and other carriers suitable for use in manufacturing preparations, in solid, semisolid or liquid form and in addition auxiliary, stabilizing, thickening and coloring agents and perfumes may be used.

Solid Compositions. For preparing solid compositions such as tablets or capsules, the principal active ingredients are mixed with a pharmaceutical carrier (*e.g.*, conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums) and other pharmaceutical diluents (*e.g.*, water) to form a solid preformulation composition containing a substantially homogeneous mixture of a composition of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. When referring to the preformulation compositions as substantially homogenous, it is meant that the active ingredients are dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of appropriate amounts.

The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings such materials including a number of polymeric acids and mixtures of polymeric acids with such materials

as shellac, cetyl alcohol and cellulose acetate. The active compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

Inhalants. For intranasal administration or administration by inhalation, the active compounds are conveniently delivered in the form of a solution or suspension from a pump spray container that is squeezed or pumped by the patient, or as an aerosol spray presentation from a pressurized container or nebulizer, with the use of a suitable propellant (*e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of an active compound and a suitable powder base such as lactose or starch.

Thapsigargin treatment leads to acute elevations of cytosolic calcium concentrations in a wide variety of cell types (Hofer and Machen, Proc. Nat. Acad. Sci. 90:2598-2602 (1993)). Since release of calcium from intracellular stores acts as a second messenger controlling an enormous list of critical cellular processes, including muscle contraction, hormone secretion and neuronal communication (Berridge, Mol. Cell. Endocrin. 98:119-24 (1994)) it is perhaps surprising that thapsigargin is so well tolerated when administered in nebulized form. The chemical structure of thapsigargin includes 3 ester groups (Christensen et. al, FEBS Lett. 335:345-348 (1993)). The cytoplasm of most eukaryotic cells is richly endowed with non-specific esterase activity, which has been shown to rapidly de-esterify xenobiotic compounds that enter the cells by diffusion (Tsien *et al.*, J. Cell Biol. 94:325-334 (1982)). It is likely, therefore, that after entering airway epithelial cells by diffusion across their apical membranes, thapsigargin is modified by the esterase activity. Loss of the ester groups reduces thapsigargin's efficacy as a calcium pump inhibitor by at least 40-fold (Christensen *et. al.*, supra). Thus thapsigargin may possess the desirable pharmacologic characteristic of being converted at its target organ into an inactive metabolite.

If this is indeed the case, thapsigargin can be applied locally to the airway by aerosol inhalation and does not diffuse out of the airway epithelial cells to enter the systemic circulation in a bioactive form. Future derivatives that exploit this feature might be even less likely to exhibit systemic toxic side effects. It is also interesting to note that

no toxicity may be associated with at least some compounds that should mimic the desired thapsigargin effect. No animal toxicity has been attributed to DBHQ, a compound that shares thapsigargin's ability to inhibit ER Ca-ATPase activity. (Chao *et al.*, J. Clin. Invest. 96:1794-1801 (1995)).

Finally, other classes of compounds in addition to calcium pump inhibitors are also likely to be of potential therapeutic utility in treating clinical conditions associated with ER retention of mis-folded proteins. Any compound which directly inhibits the function of the ER retention chaperone machinery or which alters the environment of the ER lumen so that these proteins can not function properly may possess potential clinical value.

Liquid Forms. The liquid forms, in which the novel composition of the present invention may be incorporated for administration orally or by injection, include aqueous solution, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil as well as elixirs and similar pharmaceutical vehicles. Suitable dispersing or suspending agents for aqueous suspensions include synthetic natural gums, such as tragacanth, acacia, alginate, dextran, sodium carboxymethyl cellulose, methylcellulose, polyvinylpyrrolidone or gelatin.

Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for reconstitution with water or other suitable vehicles before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.,* sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters or ethyl alcohol); preservatives (*e.g.,* methyl or propyl p-hydroxybenzoates or sorbic acid); and artificial or natural colors and/or sweeteners.

Buccal Administration. For buccal administration, the composition may take the form of tablets or lozenges formulated in conventional manners.

The active compounds may be formulated for parenteral administration by injection, which includes using conventional catheterization techniques or infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules, or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing, and/or dispersing agents. Alternatively, the active ingredients may be in powder form for reconstitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.